Role of Spirulina in protection against Cyclosporine-A induced nephrotoxicity in albino rats

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بسم الله الرحمن الرحيم

﴿ قَالُواْ سُبْحَانَكَ لاَ عِلْمَ لَنَا إِلاَّ مَا عَلَّمْتَنَا إِنَّكَ أَنتَ الْعَلِيمُ الْحَكِيمُ ﴾

صدق الله العظيم

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Contents

Introduction

The kidney is the major site for removal of toxic drugs and their reactive metabolites through urinary excretion. So, the kidney is routinely exposed to high concentration of these drugs and their active metabolites, leading to the manifestations of nephrotoxicity. The kidney, rich in vasculature, is capable of accumulating nephrotoxins **(Lim et al., 2009).**

As many drugs cause nephrotoxicity directly or indirectly, the clinical use of several life-saving drugs has been limited. Cyclosporine-A (CsA) is a potent immunosuppressive agent that is widely used to prevent cases of acute or chronic organ rejection in transplanted liver, kidney and heart **(Kewon et al., 1992).** Cyclosporine-A also protects and increases the survival time of grafts more than other immunosuppressive regimens **(Doyle et al., 1999)**. It has the advantage over other immunosuppressive drugs in lacking the depressant effects on the bone marrow **(Rang et al., 1996)**. Patients under CsA treatment are less prone to infection because cyclosporine-A does not depress haemopoiesis or affect phagocytic function **(Jones and Voorhees, 1996)**.

However, the beneficial effect of immunosuppression of cyclosporine-A is limited due to the nephrotoxicity and hypertension which are the main side effects of the drug **(Morales et al.,2001)**.

Naturally occurring antioxidants of medicinal plant origin have been tested for their protective effect against cyclosporine-A-induced nephrotoxicity. Among several known medicinal plants at present, spirulina, a microscopic filamentous blue-green alga, is emerging as a promising therapeutic aquatic microphyte. Among several occurring species of spirulina, the most commonly used in nutritional supplements is spirulina platensis (also called *Arthrospira platensis*) **(Miranda et al., 1998).**

Spirulina adds to the list of the most enriched nutrient foods currently known. The alga is rich in essential nutrients including macronutrients (proteins, lipids, carbohydrates), minerals such as calcium, iron, sodium, potassium, vitamin A (as β-caroten), vitamin K, vitamin B-12, γ linolenic acid, linoleic acid, carotenoids, phytopigments including chlorophyll and the characteristic phycobilin pigments (C-phycocyanin) in a greater bioavailable state. The pigments, C-phycocyanin and chlorophyll, give spirulina their bluish tinge, and hence the species are classified under blue green algae **(Kulshreshtha et al., 2008)**.

Spirulina and its active constituents have specific therapeutic uses beyond general nutritional values. In *vitro* and in *vivo* studies have shown that either spirulina or its active constituent, C-phycocyanin has an anti-inflammatory action **(Remirez et al., 2002)**, antioxidant action **(Miranda et al., 1998)**, and immunomodulation **(Hayashi et al., 1996)**. It has a role also in protection against chemical-and drug-induced toxicities **(Khan et al., 2006)**. Spirulina is promising in chemoprevention and cancer protection **(Schwartz et al., 1988)**, neuroprotection **(Wang et al., 2005)**, cardiovascular protection, hepatoprotection **(Gonzalez et al., 1999)** and used also as antiviral **(Ayehunie et al., 1998)**.

Aim of the work

The present work was done to study the toxic effect of cyclosporine-A on the renal tissue of adult male albino rats and to assess the possible protective role of spirulina against nephrotoxicity.

Moreover, the study aimed to determine the reversibility of nephrotoxicity after withdrawal of cyclosporine-A.

Review of Literature

Microscopic Structure of the Kidney

Verlander (1998) studied the structure of the rat kidney parenchyma and found that it is divided into 2 regions, the cortex and the medulla. The cortex is the outer portion of the parenchyma that contained the glomeruli and renal tubules. The medulla is located deeper to the cortex with no glomeruli. The medulla is divided into outer and inner regions, defined by the presence of the thick ascending limb of Henle’s loop in the outer medulla and their absence in the inner medulla. The outer medulla is divided into outer and inner stripes, defined by the presence of proximal tubule profiles in the outer stripe. The inner medulla is arbitrarily divided into **2** or **3** regions. The first region is located between the outer medulla and the papilla. The papilla contains the terminal portion of the inner medulla and can be subdivided into **2** regions, the proximal half of the papilla and the distal half, including the papillary tip.

Dwyer and Schmidt-Nielsen (2003) mentioned that in the kidney with many papillae, such as human kidney, each papilla is surrounded by a funnel-shaped calyx. This corresponds to what is known as the pelvis in the kidney with only one papilla. In the human kidney, the pelvis of the kidney is the compartment between the calyces and the ureter. In unipapillate kidney, the pelvis is a direct extension of the ureter.

Pannabecker et al. (2004) reported that the rat kidney is unipapillate with only one calyx, and it has specialized fornices which are long evaginations of the renal pelvis with epithelium similar to that of the collecting ducts. These fornices are in close association with the loop of Henle. The authors added that the medullary renal pyramid is well developed, including a strong zonation of vascular and tubular elements. The glomerulus is lobulated containing limited number of connective tissue cells between the vessels near the vascular pole. The wall of the Bowman's capsule is thin and lined with squamous epithelium that might become slightly thicker at the urinary pole.

Tryggvason and Wartiovaara, (2005) mentioned that the functional unit of the kidney is the uriniferous tubule, a highly convoluted structure. This tubule consists of two parts, each with a different embryological origin, the nephron and the collecting tubule. There are two types of nephrons: shorter cortical nephrons, subdivided into two groups, superficial and midcortical nephrons, neither of which extends deep into the medulla, and the longer juxtamedullary nephrons, whose renal corpuscles are located in the cortex and whose tubular parts extend deep into the medulla. The nephron is composed of renal corpuscle, proximal and distal convoluted tubules and loop of Henle. The nephron begins as a distended, blindly ending invaginated region of the tubule, known as Bowman's capsule, that contains arterial capillary tufts (glomerulus), and is responsible for filtration of the blood. The Bowman's capsule is lined with a single epithelial cell layer known as parietal epithelial cells. The modified cells of the inner, visceral layer are known as podocytes that wrap around the glomerular capillaries.

Gartner and Hiatt (2007) stated that with the light microscope, the proximal convoluted tubule (PCT) is composed of a simple cuboidal type of epithelium with an eosinophilic, granular-appearing cytoplasm. Paraffin sections usually display mostly occluded lumina; basally placed nuclei per cross section of the tubule; and a lack of distinct lateral cell membranes. The cuboidal cells lie on a well-defined basement membrane, easily demonstrated by the periodic acid-Schiff

(PAS) reaction. At the distal end of the proximal tubule there is an abrupt transition to the flat epithelium of the thin descending limb of the loop of Henle. Then the descending limb joins the thin ascending limb or directly joins the thick ascending limb. Distal to it is the distal convoluted tubule (DCT). In paraffin sections, the lumina of distal convoluted tubules are wide-open, the granular cytoplasm of the low cuboidal lining epithelium is paler than that of proximal convoluted tubules, and because the cells are narrower, more nuclei are apparent in tubular cross section. Because distal convoluted tubules are much shorter than proximal convoluted tubules, any section of the kidney cortex presents many more cross sections of proximal convoluted tubules than cross sections of distal convoluted tubules.

Standring (2008) described the juxtaglomerular apparatus, being adjacent to the glomerulus and composed of the afferent and efferent arterioles, the extraglomerular mesangial region, and the macula densa of the same nephron. The macula densa is a plaque of specialized tall and narrow epithelial cells. The DCTs distal to the macula densa then join each collecting duct, which is composed of a simple cuboidal epithelium. The collecting ducts descend from the cortex to the medulla. As they descend, several collecting ducts merge to form the ducts of Bellini. These ducts, then deliver the urine formed by the uriniferous tubule to the intrarenal passage, namely, the minor calyx, to be drained into a major calyx and then into the pelvis of the ureter.

El-gammal et al., (2010) mentioned in their study that the light microscope examination sections in albino rats' renal cortex revealed the presence of renal corpuscles and convoluted tubules. The renal corpuscles consist of lobulated glomeruli and Bowman's capsules with their visceral and parietal layers. The outer parietal layer is formed of flat cells while the inner visceral layer is closely applied to the glomerular capillaries. Proximal convoluted tubules with their lining cuboidal epithelial cells are seen resting on a basement membrane. They possess numerous tightly packed apical microvilli. Distal convoluted tubules are also found with their lining cuboidal epithelial cells resting on a basement membrane. In semithin sections mesangial cells are also found between loops of capillaries in addition to PCTs, DCTs and peritubular capillaries.

Mossalam et al., (2011) stated that examination of ultrathin sections of the kidney of albino rats revealed that the loops of capillaries of the glomeruli are lined by a thin layer of fenestrated endothelium. The podocyte primary processes give rise to numerous secondary foot processes resting on glomerular basement membrane. Also, the thickness of basement membrane is uniform. The proximal convoluted tubules (PCTs) are characterized by its narrow lumina. Their large cubical cells have prominent apical microvilli. The cytoplasm contains many pyknotic vesicles and numerous scattered mitochondria. Their nuclei are rounded and basally located. The distal tubules lack the prominent microvilli and the nuclei of its cells tend to bulge into the lumen with fewer mitochondria.

Hosoyamada and Sakai (2012) distinguished four types of interstitium in the rat’s kidney: the peritubular interstitium, periarterial connective tissue, glomerular and extraglomerular mesangium, and medullary interstitium. The periarterial connective tissue surrounds the intrarenal arterial tree up to the glomerular hilum, and contains lymphatic ducts and sympathetic nerve fibers. Electron microscopic observations have revealed that the periarterial connective tissue contains moderate amounts of collagen fibrils, which is suitable for the mechanical support, whereas the peritubular interstitium possesses few of such fibrils. The mesangial cells, and their